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The Human Leukocyte Platelet-activating Factor Receptor

cDNA CLONING, CELL SURFACE EXPRESSION, AND CONSTRUCTION OF A NOVEL EPITOPE-BEARING ANALOG*

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Dieter Kunzt, Norma P. Gerards, and Craig Gerards

From the Department of Pediatrics and the Ina Sue Perlmutter Laboratory, Children's Hospital, the Department of Medicine, Beth Israel Hospital, and the Thorndike Laboratory, Harvard Medical School, Boston, Massachusetts 02115

A human myeloid transcript of approximately 4 kilobases was cloned as a cDNA from an expression library based on homology with the guinea pig cDNA recently described by Honda et al. (Honda, Z-I., Nakamura, M., Miki, I., Minami, M., Watanabe, T., Seyama, Y., Okado, H., Tok, H., Ito, K., Miyamato, T., and Shimizu, T. (1991) Nature 349, 342-346) as a receptor for platelet-activating factor (PAF). The cloned DNA confers high affinity binding sites for plateletactivating factor when transfected into COS-7 cells and has binding and desensitization properties similar to the human leukocyte receptor. Southern analysis using this cDNA indicates that the PAF receptor gene is present as a single copy in the human genome. The deduced protein sequence predicts seven hydrophobic regions for the PAF receptor, characteristic of the rhodopsin gene family, and is 83% identical to the deduced protein sequence of the corresponding guinea pig molecule. A modified human PAF receptor cDNA was constructed by inserting an additional 30 nucleotides after the 5'-ATG, encoding the amino acid sequence MDYKDDDDKEF, which is specifically recognized by a monoclonal antibody. The modified cDNA encodes a functional PAF receptor and is detected by antibody on the membrane of transfected COS-7 cells. The use of this construct supports the structural model for the rhodopsin-like superfamily of receptors which places the NH2-terminal sequence on the extracellular side of the membrane, and should additionally be useful for affinity purification of the receptor protein.

The phospholipid, platelet-activating factor (PAF), displays a wide variety of biological functions in vivo, including

The nucleotide sequence(s) reported in this paper has been submitted to the GenBankTM/EMBL Data Bank with accession number(s) M76674.

‡ Current address: Dept. of Pharmacology, Biocenter of the University of Basel, Klingelbergstrasse 70, CH-4056 Basel, Switzerland.
§ To whom correspondence should be addressed: Pulmonary Div., Dept. of the Pediatrica, Children's Hospital, 320 Longwood Ave., Boston, MA 02115. Tel.: 617-735-6174; Fax: 617-730-0422.

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The abbreviations used are: PAP, platelet-activating factor; kb, kilobase; PCR, polymerase chain reaction.

granulocyte activation and chemotaxis, platelet activation, enhancement of vascular permeability, smooth muscle contraction, bronchospasm, and hypotension (1-4). These anaphylactoid actions occur in response to nanomolar quantities of PAF, and are mediated by specific receptors which are pharmacologically coupled to GTP-binding proteins (5-7). Pharmacologic studies with antagonist compounds and binding parameters suggest that there may be multiple receptors for PAF (8, 9).

Platelet-activating factor is known to be made up of a complex group of structurally related phospholipids characterized by an ether linkage at the sn-1 position, an acetyl moiety at sn-2 and a choline head group at sn-3 (10-12). Variations in the alkyl chain length have been observed or synthesized at both the 1 and 2 positions, and there are stereospecific requirements for biological function as well. No pharmacologic subclassification of PAF receptors has arisen from structure-activity studies with PAF analogs.

Recently Honda et al. (13), reported the molecular cloning of a guinea pig lung receptor for PAF identified by expression in Xenopus occytes. Because of questions arising in the human system relating to the number and identity of PAF receptor(s), we screened a U937 myeloid cell expression library using a probe prepared from guinea pig lung cDNA. In this article, we report the cloning and expression of a high affinity human receptor for PAF, and present immunohistochemical data on the topology of the receptor in transfected cell membranes.

EXPERIMENTAL PROCEDURES

Materials—Reagents and enzymes used in the construction of cDNA were products of Boehringer Mannheim. pCDM8 vector and Recherichia coli MC1061/p3 were the gift of Dr. Brian Seed, Harvard Medical School. PCR reagents and the thermal cycler were products of Perkin-Elmer Cetus. WEB2086 was provided by Boehringer Ingleheim. COS-7 cells were obtained from the American Type Culture Association. [*H]Hexadecyl-PAF (60 Ci/mmol) was obtained from Du Pont-New England Nuclear. Northern and Southern hybridizations were performed with GeneScreen Plus membranes using protocols recommended by Du Pont-New England Nuclear. Anti-Flag M5 antibody was a generous gift from Immunex. Peroxidase-labeled anti-mouse IgG was a product of Vector Laboratories.

Molecular Cloning of a Myeloid PAF Receptor—Oligonucleotides corresponding to the 5'- and 3'-coding sequences for the guines pig PAF receptor were prepared containing BamHI sites (5'-CCGGATC CGAGCCATGGAGTTAAACAGA-3', sense; 5'-CCGGATCCAGCA AGCAGCAACTAATT-3', antisense). Guines pig lung total RNA (2 µg) (14) was reverse transcribed with 20 units of avian myeloblastosis virus reverse transcriptase and 1 µg of oligo(dT) primer (15). This mixture was next added to a PCR reaction mixture containing 1 µM each of the sense and antisense primers described above, according to the recommendations provided with AmpliTaq DNA polymerase. Five cycles of PCR were performed with oligonucleotide hybridization at 45°C, and 30 subsequent cycles performed at 55°C. The band at approximately 1 kb was isolated following agarose gel electrophoresis

This work was supported by National Institutes of Health Grant HL 36162. While this manuscript was in review, Nakamura et al. (34) and Ye et al. (35) also reported cDNA sequences for the human PAF receptor. The sequences reported are identical to the one reported herein, with the exception of a T → C substitution at nucleotide 471 which makes no change in the amino acid. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

of a portion of the reaction mixture (GeneClean, Bio101, La Jolla, CA), and was labeled using random primers and [*2P]dCTP. This probe was used to screen a U937 cDNA library constructed in the expression vector pCDM8 as previously described (16). The colonies were hybridized under conditions of low stringency using 25% formamide, 5 x SSC, 1 x Denhardt's solution, and 0.1% sodium pyrophosphate at 37 °C for 18 h. Filters were washed in 2 × SSC at room temperature for 30 min with three exchanges and autoradiograms exposed for 24 h at -70 °C using an image intensifying screen. Positively hybridizing colonies in the primary library were purified and characterized. A clone, CS1, was identified by sequence analysis to contain a full length coding sequence with significant similarities to the guinea pig lung PAF receptor cDNA. This clone was extensively sequenced on both strands using the dideoxy technique with double stranded template (17). Several partial length clones were also characterized from an HL60 cell library (generous gift of Dr. Stuart Orkin, Harvard Medical School), and these sequences all yielded an unambiguous structure.

Genomic DNA and RNA Analyses—Genomic DNA was prepared from peripheral blood leukocytes (18) and 7.5-µg aliquots were digested with restriction enzymes in 20-µl aliquots overnight at 37 °C. Samples were electrophoresed at 5 V/cm through 0.9% agarose gels in 1 × TAE (Tris acetate/EDTA) buffer and blotted to GeneScreen Plus membranes by capillary action according to the manufacturer's instructions. Hybridization was performed in 50% formamide at 42 °C overnight using 106 cpm/ml of [32P]dCTP-labeled insert of clone CS1. Final washing conditions were 0.2 × SSC and room temperature for 30 min. Autoradiographs were prepared using an image intensifying screen for 24 h at -70 °C. Polyadenylated RNA was prepared with oligo(dT)-cellulose (Collaborative Research, Bedford, MA) (19) using RNA from undifferentiated U937 cells, from U937 cells differentiated for 72 h with 1 mm dibutyryl-cAMP, or from bronchiectatic human lung parenchyma resected from a patient with cystic fibrosis. Samples of 2 µg were denatured using formaldehyde and electrophoresed through 0.9% agarose. Transfer to GeneScreen Plus membranes, hybridization, and washing were accomplished as described above. Autoradiographs were prepared using an image intensifying screen at ·70 °C for 7 days.

Construction of Flag-PAF Receptor cDNA—The Flag/pCDM8 construct was created to allow COS cell expression of Flag fusion proteins (20). A synthetic duplex oligonucleotide, 5'-AGCTTCCA GCA GCC ATG GAC TAC AAG GAC GAC GAT GAC AAA GAATTC-3', bearing a HindIII site and an EcoRI site, was ligaaed to a modified pCDM8 vector containing a pBluescript polylinker. The oligonucleotide bears the consensus sequence corresponding to the human NK-2 receptor (21) 5' to the ATG at position 15, which subsequently encodes the Flag sequence, MDYKDDDDKEF. An insert encoding the human PAF receptor was prepared by PCR using a primer which mutated the normal initiating methionine to a leucine (ATG — CTG), placing the PAF receptor sequence downstream from the initiating methionine ATG for the Flag sequence.

COS Cell Expression of the Human Myeloid PAF Receptor—COS-7 cells were plated at a density of 10° cells/10-cm dish following trypainization, and were grown for 18-24 h in Dulbecco's modified Regle's medium supplemented with nonessential amino acids, aodium pyruvate, 6 mm glutamine, and 10% fetal calf serum. Cells were transfected with 2 µg of plasmid in 3 ml of Dulbecco's modified Eagle's medium containing 10% NuSerum (Collaborative Research), 1 mM chloroquine (freshly prepared, Sigma), and 400 µg/ml of DEAEdextran (Sigma). Transfections were incubated at 37 °C in an atmosphere of 5% CO2 in air for 2.5-3 h. The cells were then shocked with 10% dimethyl sulfoxide in phosphate-buffered saline for 5-10 min at room temperature and returned to media containing 10% fetal calf serum. 72-90 h following transfection the cells were washed with binding buffer consisting of 0.15 M choline chloride, 10 mM Tris-HCl, pH 7.5, 10 mm MgCl₂, and 0.25% bovine serum albumin, and incubated in the same buffer with 2 nm H-labeled PAF and appropriate concentrations of unlabeled PAF or WEB2086 for 30 min at 4 or 22 °C. Uptake of radiolabeled ligand as a function of time was determined at 4 °C. Desensitization of binding was performed by preincubating transfected cells with increasing concentrations of unlabeled PAF for 20 min at 4 °C, washing, and subsequently incubating with 2 nm ³H-labeled PAF for 30 min at 4 °C. Cells were washed with binding buffer, solubilized with 0.5% Triton X-100, scraped into scintillation vials, and cell-associated ligand determined by scintillation counting. Nonspecific binding was determined in the presence of 10 μ M unlabeled PAF. The K_d was determined from Scatchard analysis of the data obtained from duplicate points and

three separate transfection experiments.

Immunohistochemical Staining of Flag-PAF Receptor in COS-7 Cells—COS-7 cells transfected with the Flag-PAF receptor/pCDM8 plasmid described above were incubated for 20 min at 22 °C with 0 or 3 µM PAF in binding buffer. They were subsequently incubated with 10 µg/ml of anti-Flag M5 monoclonal antibody in Tris-buffered saline containing 1 mm CaCl₂ (TBS/Ca), 3% bovine serum albumin, 0.5% normal horse serum, and 0.1% sodium azide for 1 h at 4 °C. Cells were washed 3× with TBS/Ca, fixed with 2% paraformaldehyde in phosphate-buffered saline for 15 min at 4 °C, and incubated with biotinylated horse anti-mouse IgG followed by peroxidase-labeled avidin-biotin complex as recommended by the manufacturers. Cells were stained with 0.05% diaminobenzidine and 0.01% H₂O₂ in TBS/Ca, counterstained with methylene blue, coverslipped with Permount, and photographed.

RESULTS

The guinea pig lung PAF receptor cDNA (13) was prepared by PCR and used to identify an analogous human clone by hybridization with a U937 cell expression library. The longest clone obtained, pCS1, contains a cDNA of approximately 2.8 kb which hybridizes to a single transcript in U937 cell RNA of approximately 4 kb (Fig. 1, lane A). Differentiation of the cells with 1 mM dibutyryl-cAMP for 72 h leads to an apparent decrease in abundance of this message (lane B). The same ~4-kb transcript is observed in human lung, as shown in lane C.

Analysis of genomic DNA with pCS1 under conditions of high stringency reveals a single restriction fragment hybridizing with the probe. As depicted in Fig. 2, digests using BamHI, EcoRV, HindIII, or PstI yield prominent hybridization signals. Analysis of murine genomic DNA digested with the same restriction enzymes, and hybridized and washed under conditions of high stringency reveals a similar pattern, although the fragments are not always the same size (data not shown). These data suggest that pCS1 encodes a single copy gene common to mouse and man. Prolonged exposure of Southern blots reveals a faint background of cross-hybridizing bands, which may reflect related genes (data not shown).

The cDNA and deduced protein sequences of clone CS1 are presented in Fig. 3. The longest open reading frame encodes a protein of 342 amino acids, and search of the EMBL and GenBank data base releases through 1990 revealed no closely related structures. Examination of the deduced protein sequence reveals seven hydrophobic regions interspersed with polar peptide segments characteristic of rhodopsin type G protein-dependent receptors. In contrast to all other identified members of the rhodopsin family of receptors reported to date, including the PAF receptor from guinea pig, the aminoterminal domain of the human PAF receptor does not contain an N-linked glycosylation site. An Asn-X-Thr/Ser sequence is observed at positions 169–171, and a second potential N-linked glycosylation site is found at positions 333–335 at the COOH terminus.

Clone pCS1 in the expression vector pCDM8 was trans-



Fig. 1. Northern analyses of myeloid cell and lung RNA. Two µg of polyadenylated RNA was analyzed under denaturing conditions as described under "Experimental Procedures." Lane A, undifferentiated U937 cells; lane B, U937 cells differentiated for 72 h with 1 mM dibutyryl-cAMP; lane C, human lung parenchyma. A single transcript of ~4 kb was observed to hybridize with cDNA clone pCS1 under conditions of high stringency.

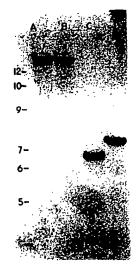


Fig. 2. Southern analysis of human genomic DNA. Samples of human genomic DNA (7.5 µg) were electrophoresed following restriction enzyme digestion with BamHI (lane A), EcoRV (lane B), HindIII (lane C), or Pst (lane D). Hybridization with radiolabeled cDNA (clone pCS1) at 42 °C with 50% formamide was followed by washing with high stringency buffer (0.2 × SSC, 24 °C, 30 min). Autoradiographs were exposed 18-24 h at -70 °C. The numerals in the left column mark the positions of standards (in kb): 12 (12.216), 10 (10.180), 9 (9.162), 7 (7.126), 6 (6.108), 5 (5.090).

fected into COS-7 cells, and the cultures examined for evidence of specific binding of 3H-labeled PAF. This cDNA confers specific binding to COS-7 cells which is not evident in nontransfected cells or in cells transfected with a different cDNA. The rate of uptake of 2 nm 3H-labeled PAF as a function of time at 4 °C is shown in Fig. 4. Under the conditions used, binding approaches equilibrium within 30-40 min. When excess unlabeled PAF is added after 20 min of incubation, approximately 50% of the radiolabeled ligand is displaced following an additional 25 min. As shown in Fig. 5A, binding is saturable, and Scatchard analysis of the data indicates a single class of binding sites with an apparent K_d of 5.3 nm (Table I). Specific binding of ³H-labeled PAF to transfected COS-7 cells is blocked by the PAF receptor antagonist, WEB2086, with an ED₅₀ of $\sim 5 \times 10^{-6}$ M (Fig. 5B), further supporting the conclusion that clone CS1 encodes a human PAF receptor. When saturation of binding experiments are performed at 4°C instead of 22°C, the calculated K, is essentially identical (5.6 versus 5.3 nm) but the apparent number of sites per cell is 8- to 10-fold lower (Table I).

Previous studies of the biological activities of PAF have shown this receptor to be very strongly desensitized to subsequent re-stimulation by ligand (22). In order to determine whether this phenomonon is an intrinsic property of the receptor, PAF receptor-transfected COS-7 cells were preincubated for 20 min at 4 °C with increasing concentrations of PAF, washed, and subsequently incubated with 2 nm ³H-labeled PAF. As shown by the data in Fig. 6, cells preincubated with as little as 1 nm PAF bind only ~30% as much ligand as untreated cells. At this concentration, however, the data presented in Fig. 5A indicate that only ~10% of the available receptor sites are bound.

We constructed a Flag-PAF receptor cDNA plasmid, in which the initiating methionine of the natural PAF receptor is mutated to a leucine by PCR and ligated to pCDM8 containing a 5' adapter encoding the Flag sequence, MDYK-DDDDKEF. The Flag sequence, including the NH₂-terminal methionine residue, is specifically recognized by a monoclonal

10 20 30 40 50 COS ATT GIT TAK AGC ATC ATC TIT GTO CTC GGG GTC ATT GCT AAT GGC TAK GTC TAT GTC TAK GT 170 180 190 200 210
THE ATG GTG AAC CTC ACC ATG GGG GAC ATG CTC THE THE ATC ACC CTC CCA CTT
PRO NET Val Are Let The HET ALS ARE HET LEU PRO LEU I IN THE LEU PRO LEU I 220 230 240 250 260 270
TOO ATT OFC TAC CAN AND CAN GOC AND TOO AND TOC COO AND THE CON TOO 330 340 350 360 370
GGC GTC ATC ACT SAT AAC GGC TTC CAG GGA GTA ACT GGG GCC ATT AMG ACT GGG
GGC GTA ATC ACT SAT AAC ACC STC CAG GGA GTA ACT GGG GCC ATT ACT GGG GCC ATC ACT GCG GCC ATC ACT GCC AT 490 500 510 520 530 540 1 GAR AGT GCT CAG GGC AAC GTC ACT CGC TGC TIT GAG CAT TAC GAG AAG GCC AAD SET ALE GLY SET GLY ASN VAI THE ATU Cys Phe Glu His Tyr Glu Lys Gly AGG GEA COLC CTC ATC ATC CAC ATC TTC ATC GTC ATC ATC CTC GTC SSO 650 670 680 690 700 | CO GTO CAG CAG CAG CAG CAG CAG CAG CTG TGG ATG GTG CCC GTO CAG CAG CAG CAG CAG CAG CAG CAG CTG TGG ATG GTG CCC GTG CAG CAG CAG CAG CAG CAG CAG CAG CAG CTG TGG ATG GTG ATT AAT GAT GCA CAT CMG GTC ACC CTC TGC CTC CTT AGC ACC AAC TGT GTC TTA Lie Aen Ado Alia Hie Gin Wal The Lem Con Lou Lem Ser The Aen Con Wal Lou GAC OCT OTT ATC TAC TOT TTC CTC ACC AND AND TAC TTC ATC AND CALC CTC ACC GAA THE RCC TTG CTT C

Fig. 3. cDNA and deduced protein sequence for the human PAF receptor. Clone pCS1 was sequenced extensively on both strands, and clones from the U937 and HL60 cDNA libraries were identical. The complete coding sequence is depicted, and the deduced protein structure contains 342 amino acids. Primers encompassing the first and last 18 nucleotides depicted are useful in PCR, yielding full length human PAF receptor cDNA.

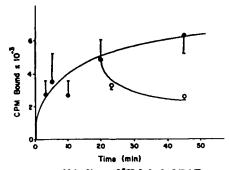


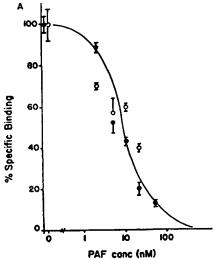
FIG. 4. Kinetics of binding of ³H-labeled PAF to transfected COS-7 cells. COS-7 cells 72 h after transfection with clone pCS1 were incubated with 2 nm ³H-labeled PAF at 4 °C as detailed under "Experimental Procedures." Each point represents the mean of duplicate determinations. Nonspecific binding was assessed by binding in the presence of 10 µM PAF.

antibody, M5, in the presence of millimolar concentrations of calcium (20). Transfection of the Flag-PAF receptor/pCDM8 construct into COS-7 cells also confers specific PAF receptor activity (Fig. 5A). Scatchard analysis suggests a slightly lower binding affinity for the ligand, but a similar number of sites per cell (Table I). Thus expression of the Flag-PAF receptor molecule is comparable to the unmodified receptor when transfected into COS cells, and is presumably oriented in a similar manner on the cell membrane. In order to confirm the model for this receptor protein, as illustrated in Fig. 7 which is based on the structure of rhodopsin, transfected cells were stained with the M5 monoclonal antibody followed by secondary antibody and horseradish peroxidase-labeled avidin-biotin complex. As indicated in Fig. 8, the epitope appears to be localized on the extracellular side of the membrane. Nontransfected cells show no staining with this antibody.

DISCUSSION

The U937 cell line is a human lymphoma clone with myeloid characteristics (23), and contains receptors for many proinflammatory mediators including C5a anaphylatoxin, the bacterial chemotaxin, formyl-Met-Leu-Phe, leukotrienes B, and D4, and interleukin-8, as well as PAF (16). Expression of many of these receptors may be modulated positively or negatively by driving differentiation of the cells towards macrophage-like cells with phorbol esters, or towards cells with the phenotype of monocytes and neutrophils with dibutyrylcAMP (24). The human PAF receptor cDNA was cloned from an undifferentiated U937 cell library by hybridization with the cDNA corresponding to the guinea pig PAF receptor (13). As indicated by the data shown in Fig. 1, differentiation of the cells towards monocytes or neutrophils appears to diminish the abundance of this mRNA. In contrast, expression of the C5a receptor is increased by treatment with dibutyrylcAMP (16).

Northern blots generated using RNAs from a number of guinea pig tissues, including lung, indicate that the guinea pig PAF receptor cDNA hybridizes with mRNAs of 2.2, 3.0, and 4.0 kb (13). Northern blots of human lung RNA, in contrast, show only a single hybridizing transcript at 4 kb, the same size as obtained with U937 cells (Fig. 1). Size fractionation of the guinea pig RNAs by sucrose density gradient centrifugation followed by expression in Xenopus oocytes indicated that the 3.0-kb transcript encodes a functional PAF receptor (13). The human PAF receptor mRNA, however, appears closest in size to the largest of the guinea pig transcripts. It is possible that the lack of expression of the 2.2- and 4.0-kb transcripts in the oocyte relates to mRNA stability or structure, or that



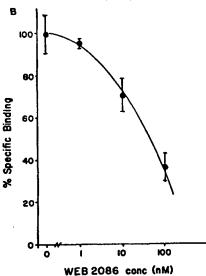


Fig. 5. A, binding of ²H-labeled PAF to transfected COS-7 cells. pCS1-transfected COS-7 cells (closed circles) or Flag-PAF receptor/pCDM8-transfected cells (open circles) were incubated with 2 nm ²H-labeled PAF and increasing concentrations of unlabeled PAF as described under "Experimental Procedures." The apparent K_c values by Scatchard analyses are presented in Table I. B, binding was competed effectively by the PAF receptor antagonist WEB2086. Each point represents the mean of duplicate determinations. Nonspecific binding was determined in the presence of 10 µm PAF.

TABLE I

Binding parameters of the human PAF and Flag-PAF receptors transfected in COS-7 cells

Values for K_d and B_{max} were determined from Scatchard analyses of displacement of binding data for each of the constructs transfected into COS-7 cells as described under "Experimental Procedures."

	Temperature	K,	B	
	•c	nM	fmol/dish	_
PAF	4	5.6	387	
****	22	5.3	2350	
Flag-PAF	22	13.5	2654	

the PAF receptor mRNA processing is different in guinea pigs and humans.

Southern blot analysis (Fig. 2) indicates that the human PAF receptor gene exists in a single copy. A similar result

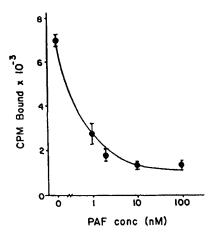


FIG. 6. PAF receptor desensitization. Clone pCS1-transfected COS-7 cells were preincubated for 20 min at 4 °C with increasing concentrations of PAF, washed, and incubated with 2 nm ³H-labeled PAF as described under "Experimental Procedures." Each point represents the mean of duplicate determinations. Nonspecific binding was assessed in the presence of 10 µM PAF and these values were subtracted from total binding to obtain the points shown.

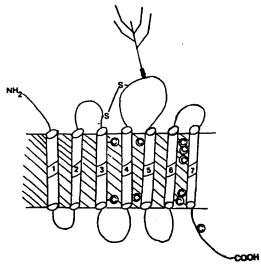


Fig. 7. Model of the PAF receptor. The rhodopsin gene family model proposes an exterior amino-terminal region, seven membrane-spanning helices, and a cytoplasmic carboxyl terminus. Special features of the PAF receptor highlighted in the Figure are 9 cysteinyl (C) residues found in the hydrophobic helices, absence of a canonical amino-terminal glycosylation site, and presence of a conserved glycosylation site in the extracellular loop between segments M4 and M5.

was obtained with Southern blots of mouse DNA, although some of the restriction fragments were different sizes. In the guinea pig, it is also possible that the multiple mRNAs observed on Northern blots reflect multiple genes for the PAF receptor in this species. As indicated previously, prolonged exposure of Southern blots of human DNA reveals multiple weakly hybridizing bands. The multiplicity of these bands and the weakness of hybridization suggest that these are related genes as opposed to additional PAF receptor genes. If multiple PAF receptors exist in humans, they may derive from alternative splicing of the mRNA.

Analysis of the deduced amino acid sequence of the human PAF receptor, as noted previously, indicates seven hydrophobic sequences alternating with more polar regions, characteristic of the seven membrane spanning protein members of the



FIG. 8. Immunohistochemical staining of the human PAF receptor transfected in COS-7 cells. COS-7 cells transfected with the Flag-PAF receptor/pCDM8 construct were stained with M5 anti-Flag monoclonal antibody, followed by biotinylated anti-mouse and avidin-biotin horseradish peroxidase, as described under "Experimental Procedures." Antibody staining of the PAF receptor is localized to the perimeter of the cell (indicated by arrows), characteristic of a cell surface epitope.

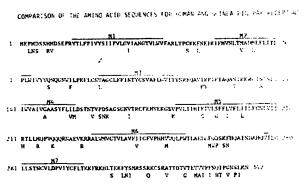


FIG. 9. Comparison of human and guinea pig PAF receptor protein sequences. The 342-amino acid human sequence is shown, with the nonidentical residues found in the guinea pig indicated. The seven predicted membrane-spanning domains are indicated by bars over the sequence.

rhodopsin family (Fig. 7) (25) The human sequence is ~83% identical to the guinea pig at the amino acid level (Fig. 9). The highest frequency of differences occurs in the COOH-terminal cytoplasmic tail with 18 residues differing among 45. A similar pattern is observed for the receptors for substance P and neurokinin A (26, 27), suggesting less genetic pressure to maintain particular amino acid sequences in this region for effective ligand binding and signal transduction.

Unique to the human PAF receptor among this class of molecules is the absence of an N-linked glycosylation site in the NH₂-terminal putative extracellular sequence (25). As noted previously, an N-linked glycosylation site occurs at positions 169-171, as it does in the guinea pig, in the putative third extracellular loop. A second glycosylation site exists at positions 333-335, in the COOH-terminal cytoplasmic tail, although this position may not be glycosylated because of its presumed intracellular location. The presence of 12 cysteine residues is also unusual for G-protein coupled receptors. Two of these residues are found in conserved positions in the first and second extracellular loops. By analogy with rhodospin (28), a disulfide bond should link residues 90 and 173. Nine of the remaining 10 cysteine residues are found in putative membrane-spanning segments. Ng and Wong (29) have reported that thiol titrants such as para-chloromercuribenzoate and N-ethyl maleimide are capable of altering the affinity of

PAF binding to its receptor. Taken together, these data suggest that there may be free thiol groups in the membrane pore created by the seven transmembrane segments and suggest a potential approach to mapping the PAF-binding site.

Another novel feature in the predicted primary structure of the human PAF receptor is the replacement of a highly conserved asparagine residue in the seventh membrane-spanning segment by aspartic acid. In nearly all G-protein-coupled rhodopsin family receptors reported to date, this asparagine residue is part of a ubiquitous region with the sequence NPXXY; indeed, we have previously exploited this homology to clone the human C5a receptor cDNA (16). Interestingly, the only other reported sequence containing an aspartic acid instead of an asparagine at this position occurs in the thromboxane A2 receptor (30). As these are the only two lipid receptors of this family whose sequences are known to date, we aligned the thromboxane A2 and PAF sequences and observed that while the protein sequences are only 19% identical, the nucleotide sequences are 50% identical. Therefore, it is conceivable that these sequences may define a new subclass of the rhodopsin family, and may be useful in low stringency screening to identify receptors for other lipid mediators, including the leukotrienes and prostaglandins.

The ligand-binding properties of the human PAF receptor, expressed in COS-7 cells, are similar to those observed using platelets and neutrophils. The K_d for the human platelet PAF receptor was reported by Hwang et al. (5) as 4.9 nm, compared with 5.3 nm for the transfected molecule (Table I), and similar to that reported for the cloned guinea pig receptor also expressed in COS-7 cells (13). Uptake of radiolabeled ligand by the receptor at 4 °C on intact COS cells (Fig. 4) was somewhat slower than that observed previously using platelet membranes (5). This may reflect the use of intact cells in our experiments. The PAF receptor antagonist, WEB2086, blocks "H-labeled PAF binding, as it does for the guinea pig molecule (13), with an ED₅₀ of 5×10^{-6} M.

Additionally, we find 8- to 10-fold higher apparent binding sites per cell when studies are performed at 22 °C instead of at 4 °C (Table I). This may reflect receptor-dependent metabolism of the ligand as described by Homma et al. (31), and by Tokumura et al. (32). Uptake is exquisitely sensitive to preexposure to PAF, since a 20-min treatment at 4 °C with 1 nm PAF, which results in occupancy of only ~10% of the available receptor sites, blocks subsequent binding of radiolabeled ligand by at least 70%. These findings may reflect internalization of the ligand-receptor complex or some other modification of the receptor (e.g. phosphorylation) which renders it unavailable for ligand binding. Indeed, the PAP receptor expressed in COS cells may be a useful system in which to study cellular utilization of PAF as facilitated by its receptor, since no significant uptake occurs on untransfected cells and nonspecific binding is very low.

Because of the utility of the Flag sequence in detection and purification of the C5a anaphylatoxin made in E. coli (20), we constructed a Flag-PAF expression plasmid with pCDM8. When transfected into COS-7 cells, the M5 monoclonal antibody, which recognizes the amino acid sequence MDYK-DDDDKEF, was used to localize the epitope to the extracellular surface, providing additional support for the rhodopsinbased model for this class of molecules. Previous support of this structure has only been provided by the β -adrenergic receptor, in which a similar approach was utilized (33). This construct should also be useful for studies of ligand-induced changes in receptor localization. Preliminary experiments indicate that PAF pretreatment appears to cause available

epitopes to polarize to one end of the cell. Additional experiments using appropriately labeled secondary antibodies or protein A can be used to extend these studies to examine such phenomena as ligand-induced metabolism of the receptor and purification of the receptor protein.

Acknowledgment-We thank Dr. Peter F. Weller, Harvard Medical School, for assistance with the immunohistochemical techniques.

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results of BLAST

BLASTN 2.2.8 [Jan-05-2004]

Reference:

Altschul, Stephen F., Thomas L. Madden, Alejandro A. Schäffer, Jinghui Zhang, Zheng Zhang, Webb Miller, and David J. Lipman (1997), "Gapped BLAST and PSI-BLAST: a new generation of protein database search programs", Nucleic Acids Res. 25:3389-3402.

RID: 1079535895-1755-55097089079.BLASTQ3

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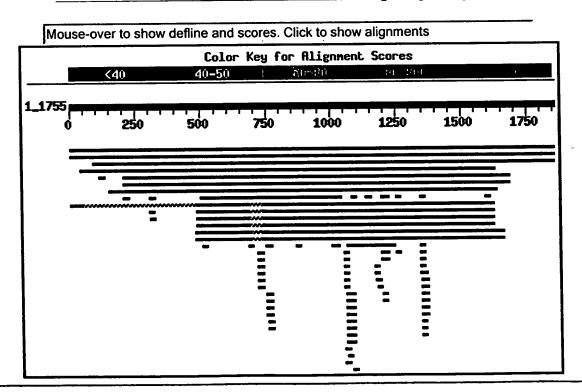
(1860 letters)

Database: All GenBank+EMBL+DDBJ+PDB sequences (but no EST, STS, GSS, or phase 0, 1 or 2 HTGS sequences)
2,102,977 sequences; 10,130,642,339 total letters

If you have any problems or questions with the results of this search please refer to the ${\tt BLAST\ FAQs}$

Taxonomy reports

Distribution of 91 Blast Hits on the Query Sequence



Sequences producing significant alignments:	Score (bits)	E Value	
<u>gi 13159911 emb AL159140.4 CNS01RGP</u> Human chromosome 14 DNA gi 15282112 emb AL162471.3 CNS01RHU Human chromosome 14 DNA	3663 3640	0.0	
gi 34190130 gb BC032831.2 Homo sapiens cDNA clone MGC:2673	3489	0.0	LU
gi 21928412 dbj AB065563.1 Homo sapiens gene for seven tra	3128	0.0	
gi 32165519 gb AY288418.1 Homo sapiens G protein-coupled r	2920	0.0	
gi 32261308 ref NM 022571.3 Homo sapiens G protein-coupled	2920	0.0	LUG
gi 2810988 gb M76676.1 HUMNPIIY20 Homo sapiens leukocyte pl	2700	0.0	LUG
gi 29611577 gb AY255588.1 Homo sapiens leukocyte platelet	<u>108</u> 2	0.0	
qi 38565931 gb BC062104.1 Mus musculus cDNA clone IMAGE:68	<u>803</u>	0.0	
<u>qi 32165533 qb AY288425.1 </u> Mus musculus G protein-coupled r <u>qi 32964966 qb AC110170.6 </u> Mus musculus chromosome 12, clon	<u>803</u> 803	0.0	
gi 32306523 ref NM 181752.1 Mus musculus G protein-coupled	<u>803</u>	0.0	LU
gi 32165543 gb AY288430.1 Rattus norvegicus G protein-coup	783	0.0	
qi 32401462 ref NM_181771.1 Rattus norvegicus G protein-co	783	0.0	
gi 32563165 emb BX004994.6 Zebrafish DNA sequence from clo	137	9e-29	LUG
gi 6031165 ref NM 001480.2 Homo sapiens galanin receptor 1	46	0.25	
gi 24648696 ref NM 169955.1 Drosophila melanogaster CG1082	46	0.25	
gi 24648694 ref NM 142709.1 Drosophila melanogaster CG1082	46	0.25	
gi 34895453 ref NM 184181.1 Oryza sativa (japonica cultiva gi 33589379 gb BT009988.1 Drosophila melanogaster RE47636	46	0.25 0.25	
gi 33589379 gb BT009988.1 Drosophila melanogaster RE47636 gi 14090356 dbj AP003233.3 Oryza sativa (japonica cultivar	46	0.25	
gi 21629409 gb AC100863.2 Homo sapiens chromosome 18, clon	46	0.25	-
gi 23171864 gb AE003734.2 Drosophila melanogaster chromoso	$\frac{46}{46}$	0.25 0.25	
gi 25140120 gb AC096709.19 Homo sapiens chromosome 18, clo gi 17861010 gb AC008308.8 Drosophila melanogaster, chromos	46	0.25	
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gi 44355566 gb AY541036.1 Homo sapiens galanin receptor 1	46	0.25	
gi 3064071 gb U90658.1 HSGALNRS1 Homo sapiens galanin recep	46	0.25	
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gi 775209 gb U23854.1 HSU23854 Human galanin receptor mRNA,	46	0.25	
gi 559047 qb L34339.1 HUMGALAREC Human galanin receptor mRN	$\frac{46}{46}$	0.25 0.25	LU
qi 12328514 dbj AP002909.2 Oryza sativa (japonica cultivar qi 22296778 qb AC121870.2 Mus musculus BAC clone RP24-121D	44	0.23	
gill3677146 gb AC013726.7 Homo sapiens BAC clone RP11-400N	44	0.98	•
gi1296091031dbi1AP005043.11 Streptomyces avermitilis genomi	44	0.98 0.98	
gi 5001541 qb AC005520.2 AC005520 Homo sapiens PAC clone RP gi 21212029 emb AL662811.20 Mouse DNA sequence from clone	44	0.98	
qi 33457241 qb AC127554.4 Mus musculus BAC clone RP24-323K	42	3.9	
gi 21618432 gb BC032702.1 Homo sapiens G protein-coupled r gi 28630143 gb AC124170.3 Mus musculus BAC clone RP23-155H	42	3.9 3.9	LUG
gi 38089509 ref XM_357908.1 Mus musculus similar to SON pr	42	3.9	
qi 37533899 ref NM 196270.1 Oryza sativa (japonica cultiva	42	3.9	U
gi 23325376 gb AE014636.1 Bifidobacterium longum NCC2705 s	<u>42</u> 42	3.9 3.9	
gi 32567975 gb AC105258.2 Oryza sativa (japonica cultivar gi 31431814 gb AE017089.1 Oryza sativa (japonica cultivar	42	3.9	
gil244180661gblAC009108.101 Homo sapiens chromosome 16 clon	42	3.9	
gi 44886087 dbj AB164051.1 Oryzias latipes cGK I beta mRNA	42	3.9 3.9	
gi 44886085 dbj AB164050.1 Oryzias latipes cGK I alpha mRN gi 29366932 gb AC009033.10 Homo sapiens chromosome 16 clon	42	3.9	

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gi|21108642|gb|AE011876.1| Xanthomonas axonopodis pv. citri...
gi|21115400|gb|AE012537.1| Xanthomonas campestris pv. campe...
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gi|21741993|emb|AL662970.2|OSJN00174 Oryza sativa genomic D...
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qi|32975800|dbj|AK065782.1| Oryza sativa (japonica cultivar...
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gi|1890580|emb|Z79692.1|RMEXPGNS R.meliloti exp gene cluster
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gi|15140691|emb|AL603645.1|RME603645 Rhizobium meliloti (Si...
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gi|24418991|emb|AL939105.1|SCO939105 Streptomyces coelicolo...
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gi|5679837|emb|AJ243961.1|OSA243961 Oryza sativa chromosome...
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gi|6634702|emb|AJ131718.1|ZMA131718 Zea mays mRNA for legum...
gi|17430467|emb|AL646076.1| Ralstonia solanacearum GMI1000 ...
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gi|10444099|gb|AF283762.1|AF283762 Mus musculus mouse-thyro...
gi|10140611|gb|AC078839.4|AC078839 Genomic Sequence For Ory...
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gi|6465842|emb|AL096774.9|HSDJ144C9 Human DNA sequence from...
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gi|1061125|emb|X83956.1|HSACCAGEN H.sapiens ACCA gene
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Alignments

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 Identities = 1772/1860 (95%)
 Strand = Plus / Plus
         cgctcctgcgtaaacacgcggttccctcggcaacgctggaacccacgtcaaaggctccgc 60
Query: 1
         Sbjct: 57931 cgctcctgcgtaaacacgcggttccctcggcaacgctggaacccacgtcaaaggctccgc 57990
         caggtccccagcgaccgccaccctccggccgagcccagctccccgcggcggccgctagc 120
Query: 61
         Sbjct: 57991 caggtccccagcgccacccctccggccgagcccagctccccgcggcggccgctagc 58050
         ccccggccccgagccaccactccgacctagcggccgcccccggtgcgggatgaggag 180
Query: 121
         Sbjct: 58051 ccccggccccgagccaccactccgacctagtggccgcccccggtgcgggatgaggag 58110
         atccgcggccgccactgggccccatggaggagccgcagccgccccaccagcgagca 240
Query: 181
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Query:	241	tggccttactgggcagccactccggcgcccctccgcggccggc	300
Sbjct:	58171	tggccttactgggcagccagcactccggcgccccctccgcggccggc	58230
Query:		cttcctccgcggccacggcggccgtgctctccttcagcaccgtggcgaccgcggcgctgg	360
Sbjct:	58231	cttcctccgcggccacggcggccgtgctctccttcagcaccgtggcgaccgcggcgctgg	58290
Query:		ggaacctgagcgacgcaagcggaggcgcacagctgccgcnnnnnnnnnn	
Sbjct:	58291	ggaacctgagcgacgcaagcggaggcgcacagctgccgctcccggtggcggccttg	58350
Query:		nnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnn	
Sbjct:	58351	gcgggtccggggcggcggcggtgaggcggcgccgctaggccggagg	58410
Query:		nnnnccgctgctgtcgcacggagctgcagtggcggcccaggcgctcgtcctcctgctca	
Sbjct:	58411	cggcgccgctgctgtcgcacggagctgcagtggcgcccaggcgctcgtcctcctgctca	58470
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Query:		ctgccgccgcggggccctggcgcgcttctgcgccgccagccgcttcttcagctcgt	
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Query:		tcgtgcggccgccgcgggagaagatcggccgccgccgcgcgctgcagctgctggcgggcg	
Sbjct:	58771	tegtgeggeegegggagaagateggeegeegeegegegetgeagetgetggegggeg	20030
Query		cctggctgacggccctgggcttctccttgccctgggagctgctcgggggcgccccgggaac	
Sbjct	58831	cctggctgacggccctgggcttctccttgccctgggagctgctcgggggcgccccgggaac	J009U

Query:		tegeggeggegeagagettecaeggetgeetetaeeggaceteeeeggaceeegegeage	
Query:	1021	tgggcgcggccttcagcgtggggctggtggtggcctgctacctgctgcccttcctgctca	1080
Sbjct: Query:		tgggcgcggccttcagcgtggggctggtggtggcctgctacctgctgcccttcctgctca tgtgcttctgccactaccacatctgcaagacggtgcgcctgtcggacgtgcgcgtgcggc	
_			
Query: Sbjct:		cggtgaacacctacgcgcgtgctgcgcttcttcagcgaggtgcgcacggccaccaccg	1200 5 9130
-		tcctcatcatgatcgtcttcgtcatctgctgctgggggccctactgcttcctggtgctgc	
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		tetggetgacetgggecaatggggecateaaceetgteatetacgccateegcaateeca	
_		acatttcgatgctcctagggcgcaaccgcgaggagggctaccggactaggaatgtggacg	1440 59370
_		ctttcctgcccagccagggcccgggtctgcaagccagaagccgcagtcgccttcgaaacc	
		gctatgccaaccggctgggggcctgcaacaggatgtcctcttccaacccggccagcggag 	
		tggcaggggacgtggccatgtgggcccgcaaaaatccagttgtacttttctgccgagagg	
		gaccaccagagccggtgacggcagtgaccaaacagcctaaatccgaagctggggatacca 	

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Query: 1741 tgatggaagattctgggggagagttgtggatttcataaagccaaacatttaaagctagag 1800
        111144544441114111141144
Sbjct: 59671 tgatggaagattctgggggagagttgtggatttcataaagccaaacatttaaagctagag 59730
Query: 1801 acgggggaggcttaccactttccccaaacaacataaaagacaatgtcccttcttcaaaag 1860
        Sbjct: 59731 acgggggaggcttaccactttccccaaacataaaagacaatgtcccttcttcaaaag 59790
                   LU Homo sapiens cDNA clone MGC:26730 IMAGE:4825908, c
>gi|34190130|gb|BC032831.2|
      Length = 2557
 Score = 3489 bits (1760), Expect = 0.0
 Identities = 1687/1772 (95%)
Strand = Plus / Plus
        gccgagcccagctccccgcgcgccgctagcccccggccccgagccaccactccgacct 148
Query: 89
        gccgagcccagctccccgcggcggccgctagcccccggccccgagccaccactccgacct 60
Sbjct: 1
Query: 149 agcggccgcccccggtgcgggatgaggagatccgcggccgccactgggccccatgga 208
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Sbjct: 61
        Query: 209
        Query: 269 cgccccctccgcggccggccacctggcgggacttcctccgcggccacggcggtgct 328
        Sbjct: 181 cgcccctccgcggccggccacctggcgggacttcctccgcggccacggcggccgtgct 240
Query: 329 ctccttcagcaccgtggcgaccgcggcgctggggaacctgagcgacgcaagcggaggcgg 388
        Sbjct: 241 ctccttcagcaccgtggcgaccgcggcgctggggaacctgagcgacgcaagcggaggcgg 300
        Query: 389
        11111111111
Sbjct: 301 cacagetgeegeteeeggtggeggeettggegggteeggggeagegegggaggeggg 360
111111111111111111111
Sbjct: 361 ggcggcggtgaggcggccgctaggcccggaggcggcgccgctgctgtcgcacggagctgc 420
Query: 509 agtggcggcccaggcgctcgtcctcctgctcatcttcctgctgtctagccttggcaactg 568
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Sbjct:			480
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Query: Sbjct:		cctgtcgctgtccctatcggatctgctcacggcgctgctctgcctgc	
Query: Sbjct:		ggacctetteacteegeeeggggtteggegeetgeegeegeegeggggeeetggegeg	748 660
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Query: Sbjct:			868 780
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		cttcttcagcgaggtgcgcacggccaccaccgtcctcatcatgatcgtcttcgtcatctg	
000	1220	ctactagagaccctactacttcctagtactactagccaccacccaqaccaqa	1288

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Query: 1649 caaacagcctaaatccgaagctggggataccagcctctaagacggttggaatggccagct 1708
      Sbjct: 1561 caaacagcctaaatccgaagctggggataccagcctctaagacggttggaatggccagct 1620
Query: 1709 tatgaaggcaaatttccactcgcattatttaatgatggaagattctgggggagagttgtg 1768
      Sbjct: 1621 tatgaaggcaaatttccactcgcattatttaatgatggaagattctgggggagagttgtg 1680
Query: 1829 caacataaaagacaatgtcccttcttcaaaag 1860
       Sbjct: 1741 caacataaaagacaatgtcccttcttcaaaag 1772
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>gi|21928412|dbj|AB065563.1| Homo sapiens gene for seven transmembrane helix rec cds, isolate:CBRC7TM_126 Length = 1590

Score = 3128 bits (1578), Expect = 0.0

Identities = 1505/1590 (94%)
Strand = Plus / Plus

Query: Sbjct:		aacccacgtcaaaggctccgccaggtccccagcgaccgccaccctccggccgagcccag	99 60
Query:			159
Sbjct:			120
Query:	160	ccccggtgcggatgaggagatccgcggccgccactgggccccatggaggagccgcagc	
Sbjct:	121	ccccggtgcggatgaggagatccgcggccgccactgggccccatggaggagccgcagc	180
Query:	220	cgcccgcccaccagcgagcatggccttactgggcagccagc	
Sbjct:	181	cgcccgcccaccagcgagcatggccttactgggcagccagc	240
Query:		cggccggccacctggcgggacttcctccgcggccacggcggccgtgctctccttcagca	
Sbjct:	241	cggccggcccacctggcgggacttcctccgcggccacggcggccgtgctctccttcagca	300
Query:		ccgtggcgaccgcggcgctggggaacctgagcgacgcaagcggaggcgcacagctgccg	399 360
Sbjct:	301	ccgtggcgaccgcggcgctggggaacctgagcgacgcaagcggaggcggcacagctgccg	
Query:		cnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnn	
Sbjct:	361		
Query: Sbjct:		nnnnnnnnnnnnnnnnnnnnnnnnnnnccgetgetgtegeacggagetgeagtggeggeee	
		·	
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Query: Sbjct:			
-		ccctatcggatctgctcacggcgctgctctgcctgcccgccc	
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Sbjct:	661		720
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Query:	820	cgttggaccgttactgcgctatcgtgcggccgccgcgggagaagatcggccgccgccgcg	879
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Query:	880	cgctgcagctgctggcggcgcctggctgacggccctgggcttctccttgccctgggagc	939
Sbjct:	841	cgctgcagctgctggcgggcgcctggctgacggccctgggcttctccttgccctgggagc	900
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Sbjct:	901	tgctcggggcgccccgggaactcgcggcggcgcagagcttccacggctgcctctaccgga	960
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Sbjct:	961		1020
Query:	1060	acctgctgcccttcctgctcatgtgcttctgccactaccacatctgcaagacggtgcgcc	1119
Sbjct:	1021	acctgctgcccttcctgctcatgtgcttctgccactaccacatctgcaagacggtgcgcc	1080
		[[[]]]]]]]]]]]]]]]]]]]]]]]]]]]]]]]]]]]	1179
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Sbjct:	1141	aggtgcgcacggccaccaccgtcctcatcatgatcgtcttcgtcatctgctgctgggggc	1200
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Sbjct	1261	cgctcctcagcgtggtggccgtctggctgacctgggccaatggggccatcaaccctgtca	1320
		tctacgccatccgcaatcccaacatttcgatgctcctagggcgcaaccgcgaggagggct	
Sbjct	: 132	l tctacgccatccgcaatcccaacatttcgatgctcctagggcgcaaccgcgaggagggct	1380
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Query: 1480 gccgcagtcgccttcgaaaccgctatgccaaccggctgggggcctgcaacaggatgtcct 1539
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        Sbjct: 1501 cttccaacccggccagcggagtggcaggggacgtggccatgtgggcccgcaaaaatccag 1560
Query: 1600 ttgtacttttctgccgagagggaccaccag 1629
        111111111111111111111111111111111111
Sbjct: 1561 ttgtacttttctgccgagagggaccaccag 1590
                   Homo sapiens G protein-coupled receptor 135 (GPR13
>gi;32165519|gb|AY288418.1|
        cds
      Length = 1485
Score = 2920 bits (1473), Expect = 0.0
Identities = 1400/1485 (94%)
Strand = Plus / Plus
Sbjct: 1
        teeggegeeecteegeggeeggeeactggeggaetteeteegeggeeacggeggee 323
Query: 264
        teeggegeeceteegeggeeggeeacetggeggaetteeteegeggeeacggeggee 120
Sbjct: 61
Query: 324 gtgctctccttcagcaccgtggcgaccgcggcgctggggaacctgagcgacgcaagcgga 383
        Sbjct: 121 gtgctctccttcagcaccgtggcgaccgcggcgctggggaacctgagcgacgcaagcgga 180
        Query: 384
        1111111111111111
Sbjct: 181 ggcggcacagctgccgctcccggtggcggcccttggcgggtccggggcagcgcgggag 240
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Sbjct: 241 gcgggggcggcggtgaggcggccgctaggcccggaggcggcgccgctgctgctgcacgga 300
Query: 504 gctgcagtggcggcccaggcgctcgtcctcctgctcatcttcctgctgtctagccttggc 563
        Sbjct: 301 gctgcagtggcggcccaggcgctcgtcctcctgctcatcttcctgctgtctagccttggc 360
Query: 564 aactgcgcggtgatgggggtgattgtgaagcaccggcagctccgcaccgtcaccaacgcc 623
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02/17/200A

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Query:	624	ttcatcctgtcgctgtccctatcggatctgctcacggcgctgctctgcctgc	683
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Sbjct:	661		720
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		ctgcgcttcttcagcgaggtgcgcacggccaccaccgtcctcatcatgatcgtcttcgtc	
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		atctgctgctgggggccctactgcttcctggtgctgctggccgccgcccggcaggcccag	
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Query	128	accatgcaggccccctcgctcctcagcgtggtggccgtctggctgacctgggccaatgg	1343

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       Sbjct: 1141 gccatcaaccctgtcatctacgccatccgcaatcccaacatttcgatgctcctagggcgc 1200
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       Sbjct: 1261 ggtctgcaagccagaagccgcagtcgccttcgaaaccgctatgccaaccggctgggggcc 1320
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Score = 2920 bits (1473), Expect = 0.0
Identities = 1400/1485 (94%)
 Strand = Plus / Plus
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Sbjct: 61
Query: 324 gtgctctccttcagcaccgtggcgaccgcggcgctggggaacctgagcgacgcaagcgga 383
       Sbjct: 121 gtgctctccttcagcaccgtggcgaccgcggcgctggggaacctgagcgacgcaagcgga 180
111111111111111111
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Query:	444	nnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnn	503
Sbjct:	241	gcgggggcggcggtgaggcggctaggccggaggcggcgctgctgtcgcacgga	300
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Sbjct:	361	aactgcgcggtgatgggggtgattgtgaagcaccggcagctccgcaccgtcaccaacgcc	420
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 Strand = Plus / Plus
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Query: 152 ggccgccgccccggtgcgggatgaggagatccgcggccgccactgggccccatggagga 211

... · ·

02/17/2004

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Query: Sbjct:		gccgcagccgccccaccagcgagcatggccttactgggcagccagc	271 120
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~~!-!

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Query: 666 ctctgcctgccgccgccttcct 688
         1111111 1111111111111
Sbjct: 403 ctctgcctacccgccgccttcct 425
>gi|32165533|gb|AY288425.1| LU Mus musculus G protein-coupled receptor 135 (Gpr13
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 Score = 803 bits (405), Expect = 0.0
 Identities = 771/893 (86%)
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Strand = Plus / Plus

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 Identities = 185/203 (91%)
 Strand = Plus / Plus
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 Identities = 45/49 (91%), Gaps = 1/49 (2%)
 Strand = Plus / Minus
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 Score = 803 bits (405), Expect = 0.0
 Identities = 771/893 (86%)
 Strand = Plus / Plus
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Query:		ctgggcttctccttgccctgggagctgctcgggggcgcccgggaactcgcggggggcag	
Sbjct:			
Query: Sbjct:		agcttccacggctgcctctaccggacctccccggaccccgcgcagctgggcgcgccttc	
Query:	1035	agcgtggggctggtggcctgctacctgctgcccttcctgctcatgtgcttctgccac	1094
Sbjct:	721	agcgtgggactggtggcttgctacttgctgcccttcctactgatgttttctgccgc	780
		taccacatctgcaagacggtgcgcctgtcggacgtgcgcgtgcggccggtgaacacctac	
		taccacatctgcaagaccgtgcgcctgtcggacgtgcgctgcggccgatgaccacttac	
Query: Sbjct:		gcgcgcgtgctgcgcttcttcagcgaggtgcgcaccggccaccaccgtcctcatcatgatc	
Ouery:	1215	gtcttcgtcatctgctgctgggggccctactgcttcctggtgctgctgctgccgcccgg	127
		caggcccagaccatgcaggccccctcgctcctcagcgtggtggccgtctggctgacctgg	
Sbjct:	961	cagggtcaggccacgcaggctccctcgctgctcaatgtggcggctgtttggctgacctgg	102
		gccaatggggccatcaaccctgtcatctacgccatccgcaatcccaacatttcgatgctc	
		•	
		ctagggcgcaaccgcgaggagggctaccggactaggaatgtggacgctttcctgcccagc 	
Query:	1455	cagggcccgggtctgcaagccagaagccgcagtcgccttcgaaaccgctatgccaaccgg	151
O	1616	- ct	157

```
Sbjct: 1201 cttqqqqcttqcaqcaggatqccttcttccaacccaqctagtgggtcaggaggggaagtg 1260
Query: 1575 gccatgtgggcccgcaaaaatccagttgtacttttctgccgagagggaccacc 1627
        Sbjct: 1261 gtcatgtgggctcgaaaaatccagttgtgctcttcttccgagagggtccacc 1313
Score = 260 \text{ bits (131)}, Expect = 9e-66
Identities = 185/203 (91%)
Strand = Plus / Plus
Query: 486 ccgctgctgtcgcacggagctgcagtggcggcccaggcgctcgtcctcctgctcatcttc 545
       Sbjct: 190 ccgctgctatggcacggggcagcggtggccgcccaggcgctcgtgctcctgctcatcttc 249
Query: 546 ctgctgtctagccttggcaactgcgcggtgatgggggtgattgtgaagcaccggcagctc 605
        Sbjct: 250 ttgctgtctagcctgggcaactgcgcggtgatgggggtgatcgtgaagcatcggcagctg 309
Query: 606 cgcaccgtcaccaacgccttcatcctgtcgctgtccctatcggatctgctcacggcgctg 665
        Sbjct: 310 cgcacggtcacaaacgccttcatcctgtcgctgtccctgtcggacctgctcactgcgctg 369
Query: 666 ctctgcctgcccgccgccttcct 688
        Sbjct: 370 ctctgcctacccgccgccttcct 392
>qi|32165543|qb|AY288430.1| LU Rattus norvegicus G protein-coupled receptor 135 (
        complete cds
       Length = 1374
 Score = 783 \text{ bits } (395), Expect = 0.0
 Identities = 797/931 (85%)
 Strand = Plus / Plus
Query: 733 cggggccctggcggcttctgcgccgccagccgcttcttcagctcgtgcttcggcatcg 792
         Sbjct: 419 cggggccctggcgcagcttctgcgccgccagccgcttcttcagttcgtgtttcggcatcg 478
Query: 793 tgtccacgctcagcgtggcgctcatctcgttggaccgttactgcgctatcgtgggccgc 852
         Sbjct: 479 tctccacgttcagcgtggcgctcatctcgctggaccgctactgcgccatcgtgcggccgc 538
```

Query:	913	ccctgggcttctccttgccctgggagctgctcggggcgccccgggaactcgcggcggcgc	972
Sbjct:	599	cgctcggcttctccctgccctgggagctgctccgggcaccccggggagcccccgactccgc	658
Query:	973	agagettecaeggetgeetetaeeggaeeteeeeggaeeeegegeagetgggegegeet	1032
Sbjct:	659	agagcttccaccgctgcctttacagaacctccccagaccctgcgcagctgggcgccgctt	718
Query:	1033	tcagcgtggggctggtggcctgctacctgctgcccttcctgctcatgtgcttctgcc	1092
		acagcgtggggctggtggcttgctacctgctgcccttcctgctgatgtttctgcc	
		actaccacatctgcaagacggtgcgcctgtcggacgtgcgcgtgcggccggtgaacacct	
Sbjct:	779	gctaccacatctgcaagactgtgcgcctgtcggacgtgcgtg	838
Query:	1153	acgcgcgcgtgctgcgcttcttcagcgaggtgcgcacggccaccaccgtcctcatcatga	
Sbjct:	839	atgcgcgcgtgctgcgctttttcagcgaggtgcgcaccggccaccaccgtgctcatcatga	898
		tcgtcttcgtcatctgctgctgggggccctactgcttcctggtgctgctgccgccc	
		ttgtctttgtcatctgctgctggggcccctactgcttcctggtgttgttggctgctaccc	
-		ggcaggcccagaccatgcaggcccctcgctcctcagcgtggtggccgtctggctgacct	
-		ggcagggtcagaccacacaggctccctcgctgctcaatgtggcagctgtttggctgacct	
		gggccaatggggccatcaaccctgtcatctacgccatccgcaatcccaacatttcgatgc	
		gggccaatggagctatcaacccggtcatatatgccatccgcaaccctaacatttctatgt	
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		tcctaggtcgcaaccgcgaagagggatataggactagaaacatggatgtttttttgccta	1136
		gccagggcccgggtctgcaagccagaagccgcagtcgccttcgaaaccgctatgccaacc	
Sbjct:	1139	gccaaggcctaggttttcaggccagaagtcgcaatcgccttcgaaatggctgtgccaaca	1198
		ggctgggggcctgcaacaggatgtcctcttccaacccggccagcggagtggcaggggacg	l
_		ggcttggggcttgcagcaggatgccttcttccaaccccgctagtgggtcaggaggggaag	j 1258
		3 tggccatgtgggcccgcaaaaatccagttgtacttttctgccgagagggaccaccagag	,
Sbjct	: 125	tggtcatgtgggctcgaaaaaacccagttgtgctcttcttccgagaggatccaccagac	c 1310

```
Query: 1633 cggtgacggcagtgaccaaacagcctaaatc 1663
         1 11 1 111111 11111111 111111
Sbjct: 1319 cagttatggcagtctacaaacagcataaatc 1349
Score = 258 bits (130), Expect = 3e-65
Identities = 189/214 (88%)
Strand = Plus / Plus
Ouery: 481 nnnnnccqctqctqtcqcacggagctgcagtggcgcccaggcgctcgtcctcctgctca 540
            Sbjct: 185 cggcaccgctgctttggcacggggcagcagtggccgcccaggcgctcgtgctcctgctca 244
Query: 541 tcttcctgctgtctagccttggcaactgcgcggtgatgggggtgattgtgaagcaccggc 600
        Sbjct: 245 tcttcttactgtctagcctgggaaactgcgcggtgatgggggtgatcgtgaagcaccggc 304
Query: 601 agctccgcaccgtcaccaacgccttcatcctgtcgctgtccctatcggatctgctcacgg 660
        Sbjct: 305 agctgcgcacggtcaccaacgccttcatcctatcgctgtccctgtcggacctgctcactg 364
Query: 661 cgctgctctgcctgccgccgccttcctggacct 694
        11111111111 | 11111111111111 | 11111
Sbjct: 365 cgctgctctgcttacccgccgccttcctcgacct 398
                       LU Rattus norvegicus G protein-coupled receptor 135
>gi|32401462|ref|NM 181771.1|
       Length = 1374
 Score = 783 \text{ bits (395)}, Expect = 0.0
 Identities = 797/931 (85%)
 Strand = Plus / Plus
         cggggccctggcgcgcttctgcgccgccagccgcttcttcagctcgtgcttcggcatcg 792
Query: 733
         Sbjct: 419 cggggccctggcgcagcttctgcgccgccagccgcttcttcagttcgtgtttcggcatcg 478
Query: 793 tgtccacgctcagcgtggcgctcatctcgttggaccgttactgcgctatcgtgcggccgc 852
         Sbjct: 479 tctccacgttcagcgtggcgctcatctcgctggaccgctactgcgccatcgtgcggccgc 538
man ar cam a man a manamananii ii ii liini l'i
Query: 913 ccctgggcttctccttgccctgggagctgctcgggggcgccccgggaactcgcggcgcgc 972
          Sbjct: 599 cgctcggcttctccctgccctgggagctgctccgggcaccccgggagcccccgactccgc 658
```

```
Query: 973 agagettecacggetgeetetaceggaceteceeggacecegegeagetgggegeget 1032
        Sbjct: 659 agagettecacegetgeetttacagaacetececagaeeetgegeagetgggegeegett 718
Query: 1033 tcagcgtggggctggtggtggcctgctacctgctgcccttcctgctcatgtgcttctgcc 1092
         Sbjct: 719 acagcgtgggggtggtggtggttgctacctgctgcccttcctgctgatgtgtttctgcc 778
Query: 1093 actaccacatctgcaagacggtgcgcctgtcggacgtgcgcgtgcggccggtgaacacct 1152
         Query: 1153 acgcgcgcgtgctgcgcttcttcagcgaggtgcgcaccggccaccaccgtcctcatcatga 1212
         Sbjct: 839 atgcgcgcgtgctgcgctttttcagcgaggtgcgcacggccaccaccgtgctcatcatga 898
Query: 1213 tcgtcttcgtcatctgctggtggggccctactgcttcctggtgctgctggccgcccc 1272
         Sbjct: 899 ttgtctttgtcatctgctgctggggcccctactgcttcctggtgttgttggctgctaccc 958
Query: 1273 ggcaggcccagaccatgcaggcccctcgctcctcagcgtggtggccgtctggctgacct 1332
         $$1111 $13111 TILL THE TILL THE TILL TO THE STREET
Sbjct: 959 ggcagggtcagaccacacaggctccctcgctgctcaatgtggcagctgtttggctgacct 1018
Query: 1333 gggccaatggggccatcaaccctgtcatctacgccatccgcaatcccaacatttcgatgc 1392
         Sbjct: 1019 gggccaatggagctatcaacccggtcatatatgccatccgcaaccctaacatttctatgt 1078
Query: 1393 tcctagggcgcaaccgcgaggagggctaccggactaggaatgtggacgctttcctgccca 1452
         Sbjct: 1079 tcctaggtcgcaaccgcgaagagggatataggactagaaacatggatgtttttttgccta 1138
Query: 1453 gccagggcccgggtctgcaagccagaagccgcagtcgccttcgaaaccgctatgccaacc 1512
         Sbjct: 1139 gccaaggcctaggttttcaggccagaagtcgcaatcgccttcgaaatggctgtgccaaca 1198
Query: 1513 ggctgggggcctgcaacaggatgtcctcttccaacccggccagcggagtggcaggggacg 1572
         Sbjct: 1199 ggcttggggcttgcagcaggatgccttcttccaaccccgctagtgggtcaggaggggaag 1258
Query: 1573 tggccatgtgggcccgcaaaaatccagttgtacttttctgccgagagggaccaccagagc 1632
         Sbjct: 1259 tggtcatgtgggctcgaaaaacccagttgtgctcttcttccgagaggatccaccagacc 1318
Query: 1633 cggtgacggcagtgaccaaacagcctaaatc 1663
                    - (11111111 111111
         1 11 1 11111
Sbjct: 1319 cagttatggcagtctacaaacagcataaatc 1349
```

Length = 3056

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```
Score = 258 \text{ bits } (130), Expect = 3e-65
Identities = 189/214 (88%)
Strand = Plus / Plus
Query: 481 nnnnnccgctgctgtcgcacggagctgcagtggcggcccaggcgctcgtcctcctgctca 540
            Sbjct: 185 cggcaccgctgctttggcacggggcagcagtggccgcccaggcgctcgtgctcctgctca 244
Query: 541 tcttcctgctgtctagccttggcaactgcgcggtgatgggggggtgattgtgaagcaccggc 600
        Sbjct: 245 tcttcttactgtctagcctgggaaactgcgcggtgatgggggtgatcgtgaagcaccggc 304
Query: 601 agctccgcaccgtcaccaacgccttcatcctgtcgctgtccctatcggatctgctcacgg 660
        Sbjct: 305 agetgegeacggteaccaacgcetteatectategetgteectgteggaectgeteactg 364
Query: 661 cgctgctctgcctgcccgccttcctggacct 694
        11111111111 | 11111111111111 | 11111
Sbjct: 365 cgctgctctgcttacccgccgccttcctcgacct 398
complete sequence
        Length = 211945
 Score = 137 bits (69), Expect = 9e-29
 Identities = 159/189 (84\%)
 Strand = Plus / Minus
Query: 1059 tacctgctgcccttcctgctcatgtgcttctgccactaccacatctgcaagacggtgcgc 1118
                       Sbjct: 94318 tacctgcttcccttcgccctcatgtgcttctgccactacaacatctgcaaaacagtccgg 94259
Query: 1119 ctgtcggacgtgcgcgtgcggtgaacacctacgcgcgcgtgctgcgcttcttcagc 1178
          Sbjct: 94258 ctgtcggagatcagggtgcggccggtcaccacttacgcgcacctgttgcgcttctacagc 94199
Query: 1179 gaggtgcgcacggccaccaccgtcctcatcatgatcgtcttcgtcatctgctgctggggg 1238
          Sbjct: 94198 gagatgcgcaccgcgaccaccgtgctcatcatgattgtgttcagcatcttctgctggggg 94139
Query: 1239 ccctactgc 1247
           111111111
Sbjct: 94138 ccctactgc 94130
>gi|6031165|ref|NM 001480.2| LUG Homo sapiens galanin receptor 1 (GALR1), mRNA
```

NO 11 7 17 NO A

```
Score = 46.1 \text{ bits } (23), \text{ Expect = } 0.25
Identities = 32/35 (91%)
Strand = Plus / Plus
Query: 1057 gctacctgctgcccttcctgctcatgtgcttctgc 1091
            11111111111111 11111111111 11111111
Sbjct: 1395 gctacctgctgccgctcctgctcatctgcttctgc 1429
>gi|24648696|ref|NM 169955.1| LU Drosophila melanogaster CG10823-PB [Drosophila m
            (CG10823) mRNA, complete cds
          Length = 1752
 Score = 46.1 \text{ bits } (23), \text{ Expect = } 0.25
 Identities = 23/23 (100%)
 Strand = Plus / Plus
Query: 1048 tggtggcctgctacctgctgccc 1070
            111111111111
Sbjct: 1346 tggtggcctgctacctgctgccc 1368
>gi|24648694|ref|NM 142709.1| LU Drosophila melanogaster CG10823-PA [Drosophila m
            (CG10823) mRNA, complete cds
          Length = 4523
 Score = 46.1 \text{ bits } (23), \text{ Expect = } 0.25
 Identities = 23/23 (100%)
 Strand = Plus / Plus
Query: 1048 tggtggcctgctacctgctgccc 1070
            1111111111111111111111111
Sbjct: 1346 tggtggcctgctacctgctgccc 1368
>gi|34895453|ref|NM 184181.1| U Oryza sativa (japonica cultivar-group) P0037C04.21
             mRNA
          Length = 1020
 Score = 46.1 bits (23), Expect = 0.25
  Identities = 29/31 (93%)
  Strand = Plus / Minus
 Query: 1196 caccgtcctcatcatgatcgtcttcgtcatc 1226
            Sbjct: 991 caccgtcctcatcatcctcgtcttcgtcatc 961
```

Drosophila melanogaster RE47636 full insert cDNA

>gi|33589379|qb|BT009988.1| Length = 4808

```
Score = 46.1 bits (23), Expect = 0.25
Identities = 23/23 (100%)
Strand = Plus / Plus
Query: 1048 tggtggcctgctacctgctgccc 1070
          11111111111111111111111
Sbjct: 1387 tggtggcctgctacctgctgccc 1409
clone:P0037C04
        Length = 137879
Score = 46.1 bits (23), Expect = 0.25
 Identities = 29/31 (93%)
 Strand = Plus / Minus
Query: 1196 caccgtcctcatcatgatcgtcttcgtcatc 1226
           Sbjct: 73349 caccgtcctcatcatcctcgtcttcgtcatc 73319
Length = 175527
 Score = 46.1 bits (23), Expect = 0.25
 Identities = 32/35 (91%)
 Strand = Plus / Plus
            gctacctgctgcccttcctgctcatgtgcttctgc 1091
Query: 1057
            11111111111111 1111111111 111111111
Sbjct: 141358 gctacctgctgccgctcctgctcatctgcttctgc 141392
>qi|23171864|qb|AE003734.2| LID Drosophila melanogaster chromosome 3R, section 72
           complete sequence
         Length = 241480
 Score = 46.1 bits (23), Expect = 0.25
 Identities = 23/23 (100%)
 Strand = Plus / Minus
Query: 1048 tggtggcctgctacctgctgccc 1070
            11111111111111111111111
Sbjct: 66542 tggtggcctgctacctgctgccc 66520
                          D Homo sapiens chromosome 18, clone RP11-707P24, comp
>gi|25140120|gb|AC096709.19|
         Length = 199285
```

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```
Score = 46.1 bits (23), Expect = 0.25
Identities = 32/35 (91%)
Strand = Plus / Minus
            gctacctgctgcccttcctgctcatgtgcttctgc 1091
Query: 1057
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>qi|17861010|gb|AC008308.8| Drosophila melanogaster, chromosome 3R, region 93C-9
            BACR10M16, complete sequence
         Length = 195868
 Score = 46.1 bits (23), Expect = 0.25
 Identities = 23/23 (100%)
 Strand = Plus / Minus
            tggtggcctgctacctgctgccc 1070
Query: 1048
             1111111111111111111111
Sbjct: 140664 tggtggcctgctacctgctgccc 140642
>gi|16258972|gb|AC008309.7| Drosophila melanogaster, chromosome 3R, region 93D-9
            BACR06L13, complete sequence
         Length = 162770
 Score = 46.1 bits (23), Expect = 0.25
 Identities = 23/23 (100%)
 Strand = Plus / Minus
Query: 1048 tggtggcctgctacctgctgccc 1070
            Sbjct: 26083 tggtggcctgctacctgctgccc 26061
                           Homo sapiens galanin receptor 1 mRNA, complete cds
>gi|44355566|gb|AY541036.1|
         Length = 1050
 Score = 46.1 bits (23), Expect = 0.25
 Identities = 32/35 (91%)
 Strand = Plus / Plus
Query: 1057 gctacctgctgcccttcctgctcatgtgcttctgc 1091
           11111111111111 1111111111 111111111
Sbjct: 623 gctacctgctgccgctcctgctcatctgcttctgc 657
Length = 1796
 Score = 46.1 bits (23), Expect = 0.25
```

```
Identities = 32/35 (91%)
Strand = Plus / Plus
Query: 1057 gctacctgctgcccttcctgctcatgtgcttctgc 1091
          Sbjct: 1721 gctacctgctgccgctcctgctcatctgcttctgc 1755
>gi|1297337|gb|U53511.1|HSU53511
                              Homo sapiens galanin receptor (Gall-R) mRNA,
         Length = 1053
 Score = 46.1 bits (23), Expect = 0.25
 Identities = 32/35 (91%)
 Strand = Plus / Plus
Query: 1057 gctacctgctgcccttcctgctcatgtgcttctgc 1091
           11111111111111
Sbjct: 623 gctacctgctgccgctcctgctcatctgcttctgc 657
>qi|775209|qb|U23854.1|HSU23854 LU Human galanin receptor mRNA, complete cds
         Length = 1050
 Score = 46.1 bits (23), Expect = 0.25
 Identities = 32/35 (91%)
 Strand = Plus / Plus
Query: 1057 gctacctgctgcccttcctgctcatgtgcttctgc 1091
           Sbjct: 623 gctacctgctgccgctcctgctcatctgcttctgc 657
>gi[559047|gb]L34339.1|HUMGALAREC LU Human galanin receptor mRNA, complete cds
         Length = 1053
 Score = 46.1 bits (23), Expect = 0.25
 Identities = 32/35 (91%)
 Strand = Plus / Plus
Query: 1057 gctacctgctgcccttcctgctcatgtgcttctgc 1091
           Sbjct: 623 gctacctgctgccgctcctgctcatctgcttctgc 657
                           D Oryza sativa (japonica cultivar-group) genomic DNA,
 >gi|12328514|dbj|AP002909.2|
            clone: P0044F08
          Length = 141528
 Score = 46.1 bits (23), Expect = 0.25
  Identities = 29/31 (93%)
  Strand = Plus / Minus
```

```
Query: 1196 caccgtcctcatcatgatcgtcttcgtcatc 1226
           1111111111111111 1111111111111111
Sbjct: 24919 caccgtcctcatcatcctcgtcttcgtcatc 24889
                          Mus musculus BAC clone RP24-121D5 from 9, complete s
>gi|22296778|gb|AC121870.2|
         Length = 191747
 Score = 44.1 bits (22), Expect = 0.98
 Identities = 22/22 (100%)
 Strand = Plus / Minus
Query: 114
            cgctagccccggccccgagcc 135
             111111111111111111111
Sbjct: 162779 cgctagccccggccccgagcc 162758
>gi|13677146|gb|AC013726.7| D Homo sapiens BAC clone RP11-400N9 from 2, complete s
         Length = 214647
 Score = 44.1 \text{ bits } (22), \text{ Expect} = 0.98
 Identities = 22/22 (100%)
 Strand = Plus / Plus
Query: 1059 tacctgctgcccttcctgctca 1080
           11111111111111111111111
Sbjct: 938 tacctgctgcccttcctgctca 959
Length = 299925
 Score = 44.1 bits (22), Expect = 0.98
 Identities = 22/22 (100%)
  Strand = Plus / Minus
            ctccgcggccacggcggccgtg 326
 Query: 305
            41111111111111111111111
 Sbjct: 61594 ctccgcggccacggcggccgtg 61573
 >gi|5001541|gb|AC005520.2|AC005520
          Length = 151123
  Score = 44.1 bits (22), Expect = 0.98
  Identities = 22/22 (100%)
  Strand = Plus / Plus
             ggaggagccgcagccgcccgc 227
 Ouery: 206
```

```
Sbjct: 28563 ggaggagccgcagccgccccgc 28584
sequence
         Length = 226642
Score = 44.1 bits (22), Expect = 0.98
Identities = 22/22 (100%)
Strand = Plus / Minus
Query: 1050 gtggcctgctacctgctgccct 1071
            11111111111111111111
Sbjct: 37854 gtggcctgctacctgctgccct 37833
>gi|33457241|gb|AC127554.4| D Mus musculus BAC clone RP24-323K23 from chromosome 8
           sequence
         Length = 178416
 Score = 42.1 \text{ bits } (21), \text{ Expect = } 3.9
 Identities = 21/21 (100%)
 Strand = Plus / Minus
Query: 719 gcctgccgccgcggggcc 739
           111111111111111111111
Sbjct: 6735 gcctgccgccgcgggggcc 6715
>qi|21618432|gb|BC032702.1| LUG Homo sapiens G protein-coupled receptor 3, mRNA
           IMAGE:5247608), complete cds
         Length = 2145
 Score = 42.1 bits (21), Expect = 3.9
 Identities = 27/29 (93%)
 Strand = Plus / Plus
Query: 1347 atcaaccctgtcatctacgccatccgcaa 1375
           111111111 111111111111 111111
Sbjct: 969 atcaaccctatcatctacgccttccgcaa 997
                           D Mus musculus BAC clone RP23-155H5 from 8, complete s
>gi|28630143|gb|AC124170.3|
          Length = 235023
 Score = 42.1 bits (21), Expect = 3.9
 Identities = 21/21 (100%)
 Strand = Plus / Minus
             gcctgccgccgcggggcc 739
 Query: 719
```

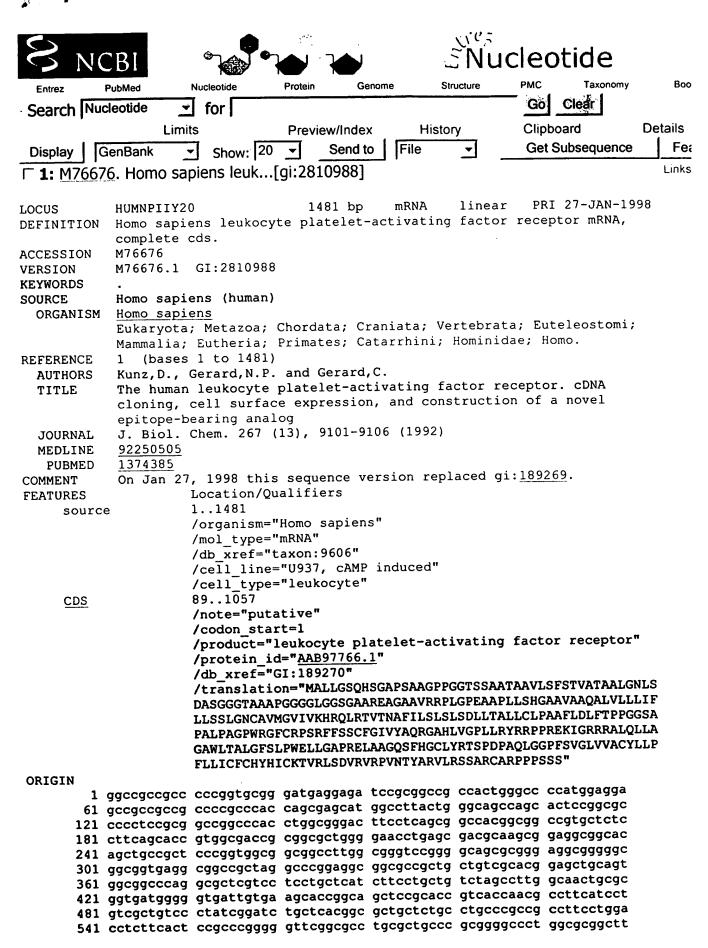
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1111111111
Sbjct: 224800 gcctgccgccgcggggcc 224780
>gi|38089509|ref|XM 357908.1| LU Mus musculus similar to SON protein (SON3) (Nega
           element-binding protein) (NRE-binding protein) (DBP-5)
           (Bax antagonist selected in saccharomyces 1) (BASS1)
           (LOC384869), mRNA
          Length = 1047
 Score = 42.1 \text{ bits } (21), \text{ Expect = } 3.9
 Identities = 21/21 (100%)
 Strand = Plus / Plus
Query: 719 gcctgccgccgcggggcc 739
           11111111111111111111111
Sbjct: 10 gcctgccgccgcggggcc 30
>qi|37533899|ref|NM 196270.1| U Oryza sativa (japonica cultivar-group) Centrin (OS
           mRNA
          Length = 2565
 Score = 42.1 bits (21), Expect = 3.9
 Identities = 24/25 (96%)
 Strand = Plus / Minus
Query: 749 cttctgcgccgccagccgcttcttc 773
            11111111111 11111111111111
Sbjct: 135 cttctgcgccgtcagccgcttcttc 111
>gi|23325376|qb|AE014636.1| D Bifidobacterium longum NCC2705 section 23 of 202 of
             genome
           Length = 11431
 Score = 42.1 \text{ bits } (21), \text{ Expect = } 3.9
 Identities = 21/21 (100%)
  Strand = Plus / Plus
 Query: 308 cgcggccacggcggccgtgct 328
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 Sbjct: 7922 cgcggccacggcggccgtgct 7942
 >qi|32567975|qb|AC105258.2| D Oryza sativa (japonica cultivar-group) chromosome 5
              complete sequence
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  Identities = 21/21 (100%)
  Strand = Plus / Plus
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```
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Sbjct: 20100 cctccgcggccacggcggccg 20120
                          D Oryza sativa (japonica cultivar-group) chromosome 10
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           of the complete sequence
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 Identities = 24/25 (96%)
 Strand = Plus / Plus
           cttctgcgccgccagccgcttcttc 773
           1111111111111111111111111111111
Sbjct: 67166 cttctgcgccgtcagccgcttcttc 67190
Length = 168656
 Score = 42.1 bits (21), Expect = 3.9
 Identities = 21/21 (100%)
 Strand = Plus / Plus
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 Strand = Plus / Minus
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Sbjct: 891 ccgtcctcatcatgatcgtct 871
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                           D Homo sapiens chromosome 16 clone RP11-140I24, compl
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 Strand = Plus / Minus
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Ouery: 682
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Sbjct: 72712 ccttcctggacctcttcactc 72692
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 Identities = 21/21 (100%)
 Strand = Plus / Plus
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Sbjct: 6287 ctggtgctgctggccgccgcc 6307
Lambda
          K
           0.711
                    1.31
    1.37
Gapped
Lambda
          K
                     1.31
           0.711
    1.37
Gap Penalties: Existence: 5, Extension: 2
Number of Hits to DB: 14,905,373
Number of Sequences: 2102977
Number of extensions: 5816
Number of successful extensions: 19
Number of sequences better than 10.0: 0
 Number of HSP's better than 10.0 without gapping: 0
 Number of HSP's successfully gapped in prelim test: 18
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 X2: 15 (30.0 bits)
 X3: 25 (50.0 bits)
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S1: 12 (25.0 bits)

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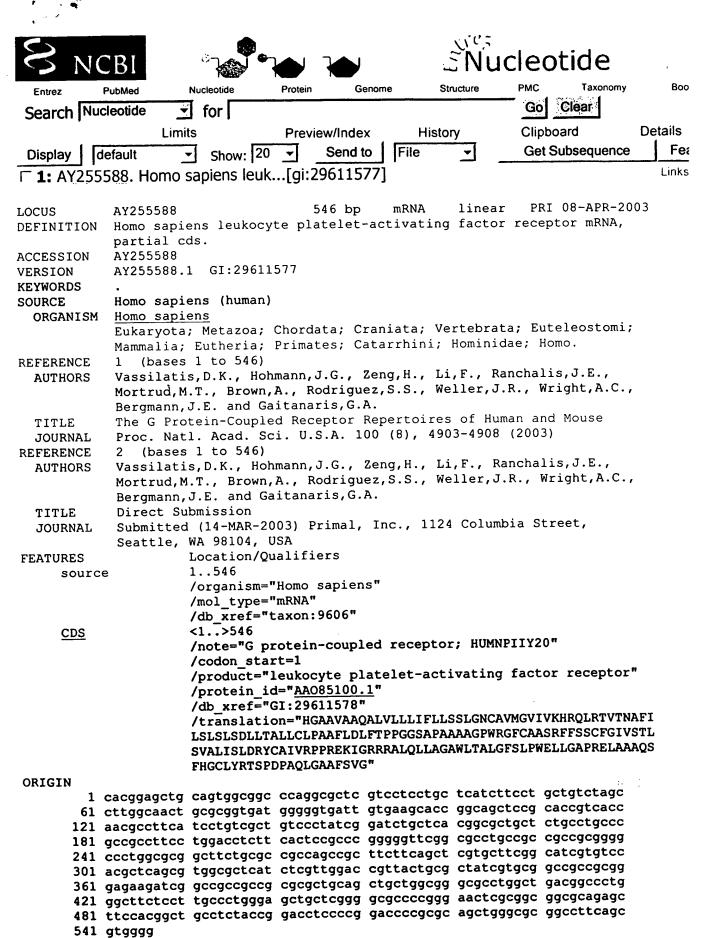


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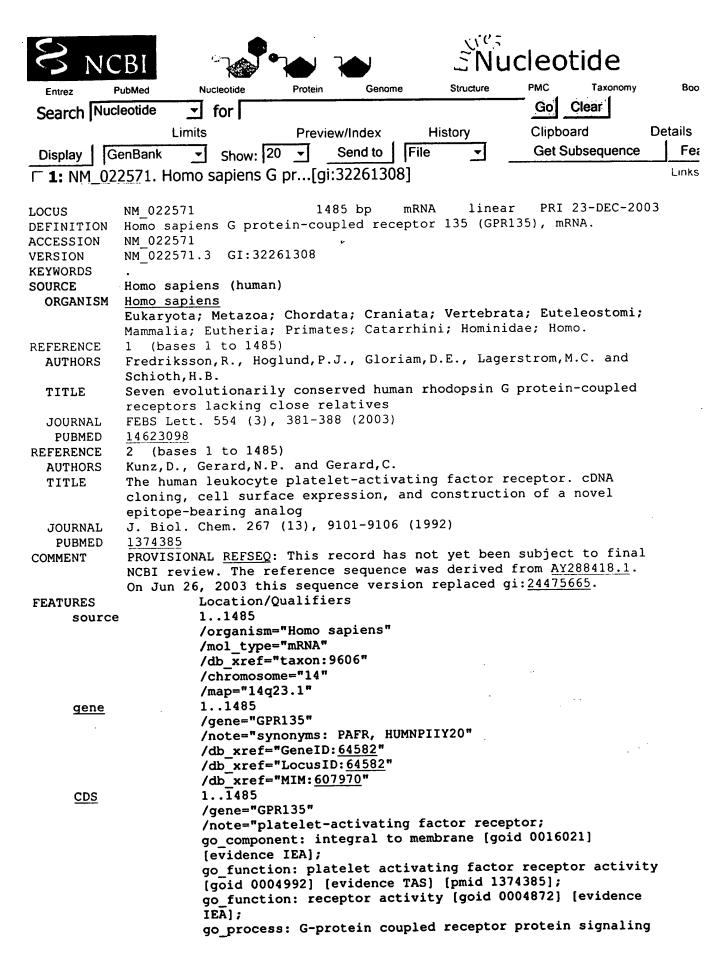
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11
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Description

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PAF receptor ·

Applications: flow

cytometry,

immunocytochemistry;

may not work for

Western blot · IgG2a ·

Clone designation:

11A4 (done 21) · PAF

is a potent

phospholipid mediator

which exerts diverse

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toll free 800.364.9897 direct dial 734.971.3335 protein coupica in receptor. The PAF receptor has been cloned from a number of species including human, rat, and guinea pig and is characterized as a 7transmembrane receptor which induces phosphoinositol turnover through Gprotein coupling.1 2 3 4 5 Northern blot analysis reveals that the receptor is expressed in leukocytes, placenta, lung, spleen, small intestine, kidney, liver, and brain.3 4 In leukocyte cell populations the receptor is found on platelets, myocytes, neutrophils, and Bcells, whereas resting T-cells and natural killer cell lines do not express the PAF receptor.6 Human monocytes treated with INF-y have a 2-6 fold increase in PAF receptor expression compared to untreated cells.7

¹ Nakamura, M., Honda, Z.,

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ızumı, 1., et al. Molecular cloning and expression of platelet-activating factor receptor from human leukocytes. J. Biol. Chem. 266, 20400-20405 (1991). ² Kunz, D., Gerard, N.P., and Gerard, C. The human leukocyte platelet-activating factor receptor. cDNA cloning, cell surface expression, and construction of a novel epitope-bearing analog. J. Biol. Chem. 267, 9101-9106 (1992). 3 Ye, R.D., Prossnitz, E.R., Zou, A., et al. Characterization of a human cDNA that encodes a functional receptor for platelet activating factor. Biochem. Biophys. Res. Commun. 180, 105-111 (1991). ⁴ Bito, H., Honda, Z., Nakamura, M., et al. Cloning, expression and tissue distribution of rat plateletactivating-factor-receptor cDNA. Eur. J. Biochem. 227, 211-218 (1994). ⁵ Honda, Z., Nakamura, M., Mikl, I., et al. Cloning by functional expression of platelet-activating factor receptor from guinea-plg lung. Nature 349, 342-346 (1991). Müller, E., Dagenais, P.,

Alami, N., et al. Identification and functional characterization of plateletactivating factor receptors in human leukocyte populations using polyclonal anti-peptide antibody. Proc. Natl. Acad. Sci. USA 90, 5818-5822 (1993). 7 Quellet, S., Müller, E., and Rola-Pleszczynski, M. IFN-y up-regulates plateletactivating factor receptor gene expression in human monocytes. J. Immunol. 152, 5092-5099 (1994). General References There are no general references associated with

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Gene

33 total records for G-Protein Coupled Receptors

Buy	Clone ID	Species	D-0-14	Gene Symbol
Γ	10H3294	Human	complement component 5 receptor 1 (CSa ligand); complement component-5 receptor-2 (CSa ligand)	CSR1
Γ	IOH12614	Human	purinergic receptor P2Y, G-protein coupled, 11	P2RY11
τ.	<u>10H22483</u>	Human	clone MGC:33224 IMAGE:5267661, mRNA, complete cds.	RDC1
τ.	EOH14039	Human	Similar to putative nuclear protein ORF1-FL49	ORF1-FL49
Γ.	10 H11484	£ Human	glycoprotein Ib (platelet), alpha polypeptide	GP18A
Ľ	80H1987	Human	tachytinin receptor 1 isoform short; RK-1 receptor; Tachytinin receptor 1 (autotance P receptor; neurolinin-1 receptor); tachytinin 1 receptor (autotance P receptor, neurolinin 1 receptor); neurolinin 1 receptor	TACRI
	80H1305	<u>s</u> Human	similar to POSSIBLE GUSTATORY RECEPTOR CLOKE PTE01	LOC11513:
C .	80H9916	Human	congulation factor II (thrombin) receptor-like 1	F281.1
Γ	80H9624	<u>t</u> Human	vescective intestinal peptide receptor 2	VSPR2
r	201106	79 Humas	endothelin receptor type A	EDMRA
C	BOH226	3Z Human	Similar to parathyroid hormone receptor 1, clone NGC:34562 24AGE:5180885, mRNA, complete cds.	PTHRI
ſ	20H135	as ttumb	Dutty blood group	· P
ſ	30H45	15 Hume	Cholecystatinin & receptor	COKER
ſ	_ 30KL1	033 Stu me	endothelial differentiation, bysophosphatidic acid G-protein-coupled receptor 4; G protein-coupled receptor; LPA receptor EDG4; Lysophosphatidic acid receptor EDG4	^{(,} EDG4
1	20H10	acc Hum	on CD97 antigen teolorun 2 procursor; feukocyte antigen CD97; seven-spen transmembrane protein	CD17
1	- 80H22	1532 Hum	 dermyl peptide receptor-like Ej lipenin A4 receptor (formyl peptide receptor related) 	* FPRLE
1	10112 2	1662 Hum	edrenomodullin receptor	ADKR
	(_ 10Hz	3232 Num	super conserved receptor expressed in broin 3	SRE63



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New Item

Novel Orphan retinal G-protein coupled Receptor (GPCR-75) selective antibodies

Anti-GPCR-75Antibodies (GPCR75-100P, GPCR75-101AP and GPCR75-112AP)

ecently a novel human G-protein coupled receptor gene has been characterized and mapped to chromosome 2p16. This gene codes for a 540 amino acid protein in retinal pigment epithelium (RPE) and cells surrounding retinal arterioles. In contrast, the Northern blot data obtained from mouse sections suggest the expression of transcripts in photoreceptor inner segments and I outer plexiform layer. The transcripts of the GPCR-75 gene (7kb) are also found in abundance in brain sections. So far, no mutations in GPCR-75 protein were identified in patients suffering from Doyne's honeycomb retinal dystrophy (DHRD), an inherited retinal degeneration disease that maps to chromosome 2p16(1).

The GPCR-75 protein is approximately 78 kDa (540 amino acids) protein that is primarily expressed in human retinal pigment epithelium (RPEs). The GPCR-75 sequence analyses suggest the presence of 7 trans-membrane domains, a characteristic feature of GPCR. The protein has putative N-glycosylation sites near the extra cellular N-terminal end of the proteins. The protein has a large 3 intra cellular loop which might be the site for interaction of G-proteins. The short carboxy terminal is intracellular and has putative post-translational modification lipid

The Anti-GPCR-75-selective antibodies were generated against conserved sequences near N- and C-termini of the protein that are unique modification sites. to GPCR-75 protein. The polyclonal antibody strongly labels a 78 kDa protein in RPE cell extracts. Anti-GPCR-75-selective antibody is also available in affinity-purified form for confocal, Western blotting and immunocytochemical analyses. FabGennix Int. Inc. will also conjugate antibodies with fluorescent probes upon request at extra charge. FabGennix Int. Inc. will also provides antibodies against proteins that are involved in retinal degenerative diseases such as various Anti-PDE antibodies, Anti-MERTK, Anti-Phospho-MERTK, EGF-containing fibulin like intracellular protein (EFEMP1), Anti-Myocilin (TIGR), Anti-Bestrophin, Anti-ELVOL4 and a Usher syndrome specific Anti-USH2a antibodies etc. FabGennix Int. Inc employs cyclic peptide methodology for generating antibodies, which results in higher titer and specificity (2). FabGennix Int. Inc., will also provide Western blot positive controls for most of these antibodies in ready-to-use buffer for easy identification of respective proteins. Limited quantities of antigens are also available. Please enquire for their availability before ordering.

			Cross reactivity	Quantity	volume	Price
Catalog #	Host Species				100 ul	\$ 195.00
	Rabbit	Polycional antisera	IR, M, H	100 m1		
GPCR75-100P		Affinity purified IgG	RMH			\$ 225.00
GPCR75-101AP	Rabbit	Amnity puttied 190	10, 44, 44	100 ug	150 ul	\$ 225.00
GPCR75-112AP	Rebbit	Milling Parimos 19	R, M, H	1		
		WB positive control	Rat	For 5 App		
PC-GPCR75	N/A	TVD positive control	n/a	250 ug	inquire	\$ 65.00
P-GPCR75	N/A	Antigenic peptides	liva	1200	1	
11 -01 -111-				an arthu		

R = set; M = mouse; H = human; C = chicken; snonk = monkey; * not all varients are labeled equally

Immunogen:

Synthetic cyclic peptide (GPCR75-101AP = PNATSLHVPHSQEGNSTS-emide; GPCR75-112AP =

STSLOBOLODLINTATLYTC-emide).

Concentration: OPCR75-101AP, GPCR-112AP IgG concentration 0.75-1.25 ang/ant in 50% entitlody stabilization buffer.

Applications:

Antibody GPCR75-100/GPCR75-101AP are ideal for WB, BABA and EHC assays. The dilutions for this antibody is for

commonly of the service the service of the second of the optimal conditions for specific essays in his/her inhoments. Distincts: WB > 1:500; immunoprecipitation & i.p pull-down essays > 1:250

Reactivity: Protocols:

This antibody detects a single 78 kDs Outher GPCR75 protein in human RPE cell extracts.

Standard protocol for various applications (WB; BADA and BEC) of this antibody is provided with the product specification sheet, however, Pub Gennix Int. Inc. strongly recommends investigators to

optimize conditions for use of this antibody in their laboratories.

Form/Storage:

The antiserum is supplied in antibody stabilization buffer with 0.02% sodium azide or thimerosal/merthiolate as preservative. The affinity-puritied antibodies are puritied on antigen-speharose affinity column and supplied as 1-1.25 mg/ml igG in antibody stabilization buffer containing preservatives with low viscosity and cryogenic properties. For long-term storage of antibodies, store at -20°C. Now these antibodies can be stored at -20°C and properties. For long-term storage of antibodies, store at -20°C. Now these antibodies can be stored at -20°C and immediately with out thewing. FebGennix inc. does not recommend storage of very distinguished. enless they are prepared in specially formulated multi use entitledy dilution buller (Cat & DiuOBuller). Working solutions of entibodies in DistOButier should be filtered through 0.45µ filter efter every use for long-term storage.



Turuclin E. B., Krischner L. S., Bellingham J., Baffi. J. Taymanas S. B., Gregor E. K., Czaky K., Stratakis C. A., Gregory-Evens C. Y. Blochem. Biophys. Res. Commun. 260, 174-180, 1999.

Farooqui, S. M., Brock. W. J., A. Hamdl., Presad. C. (1991) J. Neurochem. 57, 1363-1369.

* For users who may require large amounts of OPCR75-100P or OPCR75-101AP, please enquire about bulk material discounthis Product is for Research Use Only and is NOT intended for use in humans or clinical diagnosis.

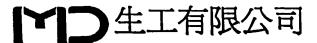
78 kDa Orphan Receptor-75 in human RPE cells. Antibody GPCR-100P (1:400)

061901-0020SF1001Z-rev10.00

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FORM: Œ Soln Œ Lyophilized. Lot # 38889S

Cat. # TR21-A, Rabbit Anti-rat TR2 Ab # 1 (affinity pure) SIZE: 100 ug

FORM: Œ Soln Œ Lyophilized. Lot # 38889A

Higher vertebrates are believed to possess at least five basic tastes: Sweet, bitter, sour, salty, and unami (the taste of monosodium glutamate). Taste receptor cells that may selectively reside in various parts of the tongue and respond to different tastants and perceive these taste modalities. Circumvallate papillae, found at the very back of the tongue, are particularly sensitive to biter substances. Foliate papillae, found at the posterior lateral edge of the tongue, are sensitive to sour and bitter. Fungiform papillae at the front of the tongue specialize in sweet taste.

Recently, two novel taste receptors, TR1 and TR2, have been cloned with distinct topographical distribution in taste receptor cells and taste buds. TRs are members of a new group of 7 TM domain containing GPCR distantly related to other chemosensory receptors (Ca+-sensing receptor (CaSR, a family of putative hormone receptor (V2R), and metabotropic glutamate receptors). TR1 is expressed in all fungiform taste buds, whereas TR2 localized to the circumvallate taste buds. Both receptors do not co-localize with gustducin.

Source of Antigen and Antibodies

TR1 (rat 840 aa) and TR2 (rat 843 aa) share ~40% homology with each other, and ~30% with CaSR, and 22-30% with V2R pheromone receptors and mGLURs. Rat TR are 7 TM domain containing protein with an extra long N-terminal, extracellular domain (1). A 19 AA Peptide (designated TR21-P; control peptide) sequence near the C-terminus of rat TR2(1) was selected for antibody production. The peptide was coupled to KLH, and antibodies generated in rabbits. Antibody has been affinity purified using control peptide-Sepharose.

Form & Storage

Control peptide Solution is provided in PBS, pH 7.4 at 1 mg/ml (100 ug/100 ul). Antiserum is supplied as neat serum (100 ul soln or lyophilized). Affinity pure antibodies were purified over the peptide-Sepharose column and supplied as 1 mg/ml soln in PBS, pH 7.4 and 0.1% BSA as stabilizer (100 ul in solution or Lyophilized).

The peptides and antibodies also contain 0.1% sodium azide as preservative. Lyophilized products should be reconstituted in 100 ul water and gently mixed for 15 min at room temp. All peptide/antibody

生工有限公司: Rat Taste Recortor 2 (TR2) Antibodies

received in solution or

reconstituted from lyophilized vials should be stored frozen at -20oC or below in suitable aliquots. It is not recommended to store diluted solutions. Avoid repeated freeze and thaw.

Recommended Usage

Western Blotting (1:1K-5K for neat serum and 1-10 ug/ml for affinity pure antibody using ECL technique).

ELISA: Control peptide can be used to coat ELISA plates at 1 ug/ml and detected with antibodies (1:10-50K for neat serum and 0.5-1 ug/ml for affinity pure).

Histochemistry & Immunofluorescence: We recommend the use of affinity purified antibody at 1-20 ug/ml in paraformaldehyde fixed sections of tissues (1).

Specificity & Cross-reactivity

The 19 AA rat TR21-P control peptide is specific for rat TR2. It has no significant sequence homology with TR1 or gustducin or pheromone receptors. Antibody cross-reactivity in various species has not been studied. The TR21-P control peptide is available to confirm specificity of antibodies.

References:

1. Hoon MA et al (1999) Cell 96, 541-555; Lindemann B (1999) Nature Med. 5, 381-382

"Neat Antisera" are the unpurified antiserum and it is suitable for ELISA and Western.
"Affinity pure" antibodies have been over the antigen-affinity column and recommended for immunohistochemical applications.

"Control peptides" can not be used for Western as they are very short peptides. They are intended for ELISA or antibody competition studies.

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